Understanding Immunesenescence.

Whittaker, Anna; Duggal, Niharika; Oyebode, Jan R.; Lord, Janet

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Chapter 7: Understanding Immunesenescence

Whittaker, A.C., Upton, J., Arora Duggal, N., Deb, C., Randhawa, C., Oyebode, J., & Lord, J.M.
University of Birmingham

Chapter Abstract
This chapter discusses the impact of hip fracture in older age and in particular factors affecting recovery of physical function and wellbeing. It focuses particularly on a study of the impact of depression following hip fracture in older adults, and the influence of this depression on a range of outcomes including immune function, stress hormones, illness perceptions, physical function, and length of stay in health service and rehabilitation facilities. It shows that depression is common following hip fracture among older people, and is the biggest predictor of a slower recovery and poorer function in terms of immunity, wellbeing, and physical ability. A pilot study associated with this research showed that illness perceptions following hip fracture did not differ between UK and Punjabi older adults, but that Punjabi speakers in India had greater levels of distress. Implications for health service policy and practice resulting from this research are discussed, including potential intervention strategies to improve outcomes after hip fracture.

Chapter Keywords: depression, hip fracture, illness perceptions, immunity, stress hormones,

1. Introduction

With the ageing of the population hip fractures are a growing issue in the UK (Dennison et al., 2006). At least half of older adults who have suffered a hip fracture never regain their previous function (Stevens and Olson, 2000), with mortality at one year recorded as high as 33% (Roche et al., 2005). The factors influencing recovery from hip fracture are poorly understood. These include depression; a common co-morbidity in these patients (Nightingale et al., 2001).

The prevalence rate for depression in people who have had a hip fracture across eight US and UK studies ranged from 9 - 47% (Holmes and House, 2000). Importantly, depression in people who have suffered a hip fracture has been associated with increased risk of infections and poor survival (Nightingale et al., 2001), impaired recovery and a reduced ability to regain pre-fracture levels of physical functioning (Mossey et al., 1990).

It is well documented that ageing is accompanied by poor functioning of the body's immune system (Panda et al., 2009, Dorshkind et al., 2009). This is called immunesenescence or immune

ageing and it contributes to the increased risk of infection in old age (Gavazzi and Krause, 2002). Particular aspects of immune ageing can be observed in specific important immune system cells. For example, neutrophils are key cells in the immune system which are responsible for providing protection against bacteria such as those that cause hospital acquired infections and pneumonia. Ageing is accompanied by a decline in neutrophil ability to ingest such bacteria (Butcher et al., 2001), and their ability to kill the bacteria once ingested (Tortorella et al., 2000). Similar reductions in efficacy have been shown in other immune cells, such as monocytes, with advancing age (Shaw et al., 2011).

An additional important component of the immune system are natural killer (NK) cells, which are cells capable of destroying cancer cells and cells infected with viruses (Farag et al., 2003). Older adults have an age-related decrease in NK cell function (Hazeldine et al., 2012), which may explain why older adults are more susceptible to cancer and virus infections such as influenza. Further, ageing is also accompanied by changes in immune cells that are very focused and kill specific infections because they have encountered them before, this is termed immune memory, or because the older adult has had a vaccination to protect them. These cells, called lymphocytes, do not function as well in older adults and this reduces the ability to benefit from vaccines in old age. In addition, some of these cells have a specific role in dampening down immune responses to prevent autoimmune disorders, these are called regulatory cells (Blair et al., 2010). Interestingly, a recent study in our group has reported an age-related decrease in the numbers and function of this type of cell which might contribute towards an increased risk of autoimmune diseases such as rheumatoid arthritis with age (Duggal et al., 2013b).

Interestingly, there is accumulating evidence suggesting that the effects of psychological stress and ageing are additive, with chronic stress worsening the effects of ageing on immunity in older adults (Kiecolt-Glaser and Glaser, 1999). For example, older adults who had suffered stressful life events, such as bereavement and marital dissatisfaction, have lower levels of antibody production in response to the annual ‘flu vaccination than those who had not experienced these stressors (Phillips et al., 2006). In addition, neutrophils are susceptible to the effects of stress, with their ability to kill bacteria reduced in older people who have suffered the trauma of a hip fracture (Butcher et al., 2005) or bereavement (Khanfer et al., 2011).

One of the ways in which stress affects bodily systems, including the immune system, is via the production of stress hormones. Stress is sensed in the brain by the hypothalamus which signals the adrenal gland to produce stress hormones which circulate in the blood and reach immune cells and influence their function (Tsigos and Chrousos, 2002). Cortisol is the main stress hormone and is a potent immune system suppressor and can also contribute to the development of depression (Fischer et al., 2017). Dehydroepiandrosterone sulphate (DHEAS), is another steroid hormone produced by the adrenal gland, and has the opposite actions to cortisol being anti-depressive (Hough et al., 2017) and immune-enhancing, including the ability to increase the bacterial killing mechanisms of neutrophils (Hazeldine et al., 2010, Radford et al., 2010).

Although cortisol levels generally may not increase with ageing, they are higher in relation to DHEAS which decreases with age in men and women (adrenopause), thus ageing is accompanied by an elevated cortisol:DHEAS ratio; this may be a key factor contributing towards age-associated immune dysfunction (Buford and Willoughby, 2008). As stress increases cortisol production, this age-related dysfunction is made worse by stress. Our group reported a raised cortisol:DHEAS ratio in older hip fracture patients versus young comparable trauma patients, and these higher cortisol levels were also accompanied by reduced neutrophil bacterial killing ability and increased incidence of bacterial infections like pneumonia (Butcher et al., 2005).

Cortisol levels are often higher in individuals with depression (Deuschle et al., 1997, Lesch et al., 1988). Higher cortisol levels in older adults have also been associated with frailty, including poor standing and walking performance (Peeters et al., 2007). Low levels of DHEAS have also been related to poorer physical function (Berkman et al., 1993). Importantly, the cortisol:DHEAS ratio is higher in older hip fracture patients than in healthy older adults (Dubin et al., 1999) and younger comparable fracture patients (Butcher et al., 2005). This hormone imbalance may thus be a major determinant of frailty in older people following hip fracture, as well as their susceptibility to infections, particularly in those with concomitant depression.

A pilot study, as part of this larger project, focused on patients' perceptions of their illness. There is evidence to support the connection between illness perceptions and outcomes in a range of conditions including chronic fatigue syndrome (Moss-Morris et al., 1996), Addison's disease (Heijmans and De Ridder, 1998) and multiple sclerosis (Jopson and Moss-Morris, 2003). Illness perceptions have been found to be related to wellbeing and mood (Murphy et al., 1999), and to participation in rehabilitation programmes (Cooper et al., 1999). Further, there is evidence that minority cultural groups hold illness perceptions that differ in various respects from the majority in the UK (Kim et al., 2012). For research into minority ethnic groups to produce useful findings, studies needs to acknowledge variations in religion and culture by working with defined groups. Consequently, these issues were also considered in the present project.

2. *Aims and Methods*

The "Synergistic effects of physical and psychological stress upon immunosenescence" NDA study sought to test whether psychological distress, specifically depressive symptoms emerging after a hip fracture, would act additively on top of the physical stress of hip fracture to amplify the effect of ageing upon immunity (immunesenescence) and physical frailty. It also examined the role of the cortisol:DHEAS ratio and cytokines (immune messengers) as potential mechanisms that might explain any of the effects on immunity and frailty.

An integrated pilot study within this overall project aimed to explore the implications of attitudes to hip fracture of a significant minority ethnic patient group in the UK, and the consequent implications for tailoring approaches to rehabilitation. Therefore in this pilot study the intention was to recruit only hip fracture patients from the Punjab region of India (excluding Punjabis from Pakistan; a different religious group).

Participants with hip fracture were 101 White British older adults of whom 81 were female. A parallel pilot study of Punjabi-speaking older adults was recruited elsewhere for comparison, as discussed below. The participants had an average age of 83.9 years and had been admitted to hospital as in-patients with a fractured neck of femur (hip fracture). Participating hospitals were all located in the West Midlands, UK. Participants were all aged 60+ years and did not have any existing medical conditions or medications that could affect the immune system, dementia, taking antidepressants or having a previous diagnosis of depression before the age of 50 years. In this way we aimed to recruit individuals who had likely developed depression post-fracture, rather than those who already had a history of depression. Fifty healthy older adults were also recruited from the community as a control group via the Birmingham 1000 Elders cohort of healthy older adults involved in research at the University of Birmingham. These controls also had to meet the criteria above but not have a hip fracture. We also involved a sub-sample of these healthy controls to advise us on the usability of the questionnaire packs and tests that were administered during the study.

For the integrated pilot study, we attempted to recruit an additional 30 Punjabi hip fracture patients concurrently with Caucasian patients from five hospitals in the West Midlands. Only one Indian Punjabi patient was recruited, who later withdrew. Our Steering Group Committee including an age- and ethnicity-relevant advisory member had considered barriers to participation in advance, and translated validated questionnaires as well as a Punjabi-speaking technician to assist with recruitment were employed, however, this did not make a difference to the poor recruitment of this sub-sample.

The main research study compared these three groups of older adults: hip fracture patients with or without depressive symptoms and healthy older adults at 6-weeks post-fracture. This is the first time that previously non-depressed patients have been assessed for emerging depression post-hip fracture. All hip fracture patients were recruited while in hospital, then completed questionnaires, structured interviews and provided a blood sample six weeks and six months after hip fracture. Control participants completed a depression and anxiety symptoms scale and basic demographic information when attending the university for blood sampling around the same time as the patients' 6 week sample. Blood samples were taken between 08.00 and 11.00 to minimise any effect of daily variations in steroid levels. None of the participants had an acute infection at the time of blood sampling. Interviews were performed either in hospital or the participants' home.

The assays for neutrophil and monocyte function, namely phagocytosis (bacteria ingesting ability) and superoxide production (bacteria killing ability) were performed on blood the same day as blood sampling. We also looked at numbers of regulatory immune cells. Serum from the blood samples from hip fracture patients and healthy control subjects were analysed for stress hormones, namely cortisol and DHEAS.

Standard socio-demographics (age, sex, occupation), health behaviour information, and all other illnesses present, and medications (prescription and over-the-counter), were recorded by the interviewer. The psychological status of the participant was assessed by means of validated psychological questionnaires. Depression was evaluated using the Geriatric Depression Scale (GDS) (Yesavage et al., 1982). Depression was defined as a GDS score greater than or equal to 6 (Sheikh et al., 1986). The Hospital Anxiety and Depression Scale (HADS) was also used to confirm the presence of depression and anxiety (Zigmond and Snaith, 1983). The Oxford Hip Score (Dawson et al., 1996) (OHS) is a 12-item questionnaire validated to assess activities of daily living (ADL) and ability in patients undergoing hip replacement surgery. Physical frailty was assessed in part by the OHS but, in addition, upper body strength was measured as handgrip strength using a hydraulic hand dynamometer, lower body strength using the Timed Up and Go (TUG) test (Podsiadlo and Richardson, 1991) which is getting up and walking speed over three metres and back, and the Berg Balance Scale (BBS) (Berg et al., 1992). The BBS comprises activities to assess balance standing and during the performance of tasks. Body mass index (BMI) was computed as kg/m^2 from measured height and weight. Health behaviours, including smoking, alcohol intake, diet, exercise, and sleep duration, were recorded using a simple questionnaire adapted from the Whitehall study (Marmot et al., 1991).

For the pilot study, given the difficulties of recruitment of Punjabi-speaking participants, an alternative strategy was developed by the Steering group. Two University of Birmingham medical students, with work placements in the Punjab region of India, agreed to collect these pilot data in India. The limitations of collecting comparative data in India rather than the UK were recognised and are discussed below. However, the study research team agreed that data from the Punjab may guide the development of future studies addressing illness perceptions of South Asians in the UK. Patients were recruited from two hospitals in the city of Jalandhar, Punjab: Orthonova Joint and Trauma Hospital and Civil Hospital. Inclusion criteria were as for the UK sample except that to facilitate recruitment, those with concurrent depression, diabetes, cancer or chronic obstructive pulmonary disease were included, and the lower age limit was 60 years for men as well as women.

Pilot study participants in India completed translated versions of the Brief Illness Perception Questionnaire (BIPQ) (Broadbent et al., 2006), Oxford Hip Score (OHS), the Hospital Anxiety and Depression Scale (HADS), and patient basic socio-demographic information. Where validated translated versions were not available, such as for the BIPQ, questionnaires were

translated into Punjabi, and independently back-translated. Discrepancies were then discussed, and agreement reached concerning the most appropriate wording.

3. *Findings*

All of our findings were published in peer reviewed journals (Duggal et al., 2014a, Duggal et al., 2015a, Duggal et al., 2014b, Duggal et al., 2013a, Duggal et al., 2015b, Phillips et al., 2015, Phillips et al., 2013). In summary, 101 hip fracture patients and 43 controls were recruited for the six week study. By six months, 66 hip fracture patients remained in the study. Withdrawals were due to death, being too unwell to be tested, being non-contactable or no longer meeting the inclusion criteria (e.g., taking anti-depressant medication). The flow of participants through the study is shown in Figure 1.

For the pilot study in India, 22 patients who met the inclusion criteria were recruited.

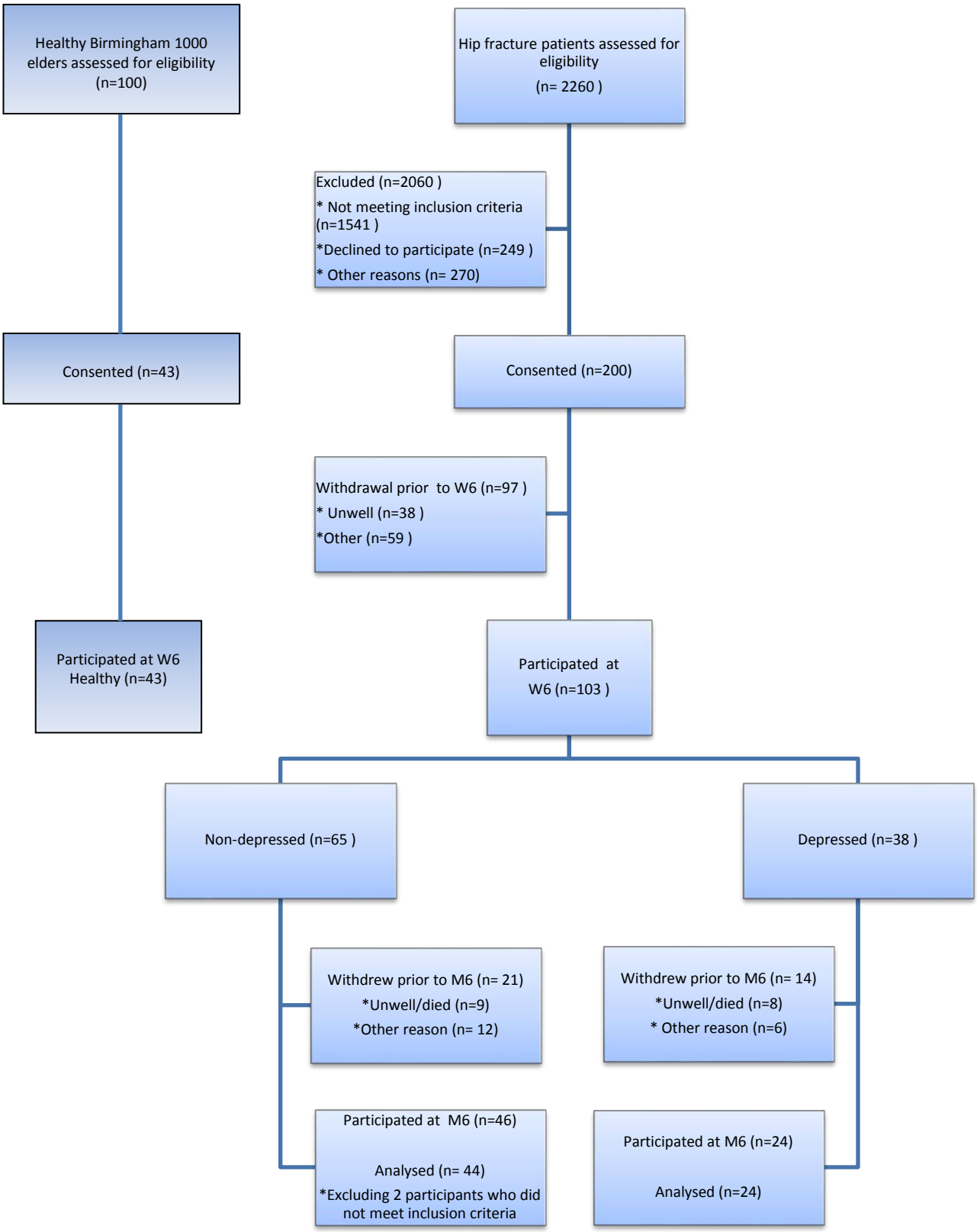

Figure 1: Flow of participants through the study (modified from (Phillips et al., 2013)

3.1 Participant characteristics

Patients were classified into two groups on the basis of their depression scores: 37% of the hip fracture patients had depressive symptoms six weeks after their fracture. These groups differed on age and Body Mass Index (BMI), such that the non-depressed group was slightly younger and had a higher BMI. However, there were no significant differences between the two hip fracture groups on other variables. At month 6, data were available for 66 hip fracture patients; 29% of whom were depressed. Participants classified as depressed had significantly higher GDS scores by an average of 5.6, at week 6. At month 6, this difference remained significant.

3.2 Length of hospital stay and infections

A range of hospital-related and community-acquired infection data were collected from patients' hospital and GP notes within the six months post-hip fracture. The depressed group spent a significantly longer entire length of stay in hospitals or rehabilitation centres, although the length of inpatient stay at the hospital their surgery took place at did not significantly differ (Phillips et al., 2015). Hip fracture patients were significantly more likely to be discharged to a rehabilitation unit, and there was a slightly increased number of readmissions to hospital (Phillips et al., 2015). However, depressed patients did not have a greater number of infections while in hospital. Although they had a slightly higher number of infections in the six months following their hip fracture, this was not statistically significant (Phillips et al., 2015). The types of infections experienced by depressed and non-depressed patients were not significantly different between groups.

Table 1: Characteristics of participants

Variable	**Hip fracture patients (HF) N = 65**	**Hip fracture patients with depressive symptoms (HF + D) N = 38**	**Healthy Controls N = 43**
Week 6	**Number / Average**		
Age (years)	83.8	84.0	74.9
Sex (Female)	58	25	26
Body Mass Index (kg/m^2)	23.5	22.7	27.2
Alcohol consumption (one or more units per week)	13	24	13
Sleep (< 8hr per night)	19	28	23

3.3 Physical performance in hip fracture patients

Physical frailty was assessed using four measures as described above. Patients with symptoms of depression were less able to engage in activities of daily living (ADL) than non-depressed patients at week six and month six although ADL significantly improved over time. Participants with depression took significantly longer to complete the Timed Up and Go (TUG) walking speed test at week six and month six, compared to the non-depressed group (Phillips et al., 2013). Both groups were far slower than age-related norms at both time-points, although walking speed improved over time in both groups. Depressed patients also scored significantly worse on the Berg Balance scale at week six compared to the non-depressed patients, but there was no significant difference by month six (Phillips et al., 2015).

3.4 Neutrophil functioning in hip fracture patients

When we compared neutrophil numbers between our hip fractured participants with and without depression and the healthy control group, significantly lower numbers were observed in healthy older adults compared with both groups of older adults with hip fracture, though all were within the normal range. Neutrophil phagocytosis (eating ability) did not differ significantly between the two groups of hip fracture patients and healthy controls. Neutrophil superoxide production (killing ability) in response to stimulation differed between our three groups, but the significant impairment was restricted to the hip fractured participants who developed depressive symptoms. Hip fracture patients with higher GDS scores had poorer neutrophil superoxide production (Duggal et al., 2013a).

3.5 Monocyte functioning in hip fracture patients

Similar to neutrophils, monocytes play a key role in removal of pathogens and providing protection against infections. We compared monocyte count between our three groups, but no significant differences were detected nor for monocyte phagocytic (eating) ability. Monocyte superoxide production (killing ability) was different between the three groups, but the significant impairment was restricted to the hip fracture patients who developed depression (Duggal et al., 2014a), similar to our neutrophil data. Further, higher depression scores related to lower monocyte superoxide production (Duggal et al., 2014a), again in the same way as depression scores related to neutrophil function.

3.6 NK cell numbers and cytotoxicity in hip fracture patients

On examining NK cells in the participants, similar to the results above, we did not find any significant differences in percentages, or absolute numbers of NK cells between our three groups. However, a significant impairment in NK cell killing capacity was restricted to the hip fractured participants with new onset depression compared to healthy controls and those

without depressive symptoms (Duggal et al., 2015a). Again, depressive symptoms predicted a reduction in NK cell killing ability, such that hip fracture patients with greater depressive symptoms had poorer NK cell activity.

3.7 Hormone imbalance in hip fracture patients with depressive symptoms

Analysis of stress hormone levels from the blood samples revealed significant differences: higher cortisol levels, reduced DHEAS levels, and an elevated serum cortisol:DHEAS ratio was observed in hip fracture patients with depressive symptoms compared to those with hip fracture alone and controls (Phillips et al., 2013). Elevated serum cortisol:DHEAS ratios have been previously reported as an indicator of individuals at risk of depression (Young et al., 2002). Here, the higher the depression scores, the higher the cortisol:DHEAS ratio. However, the hormone ratio was not related to neutrophil, monocyte or NK cell function suggesting that it is not the main underlying link between depressive symptoms and impaired immune function. Interestingly, the ratio between these hormones did relate to physical function. The cortisol:DHEAS ratio was related to walking speed as measured by the TUG at month six, such that the higher the cortisol:DHEAS ratio, the longer it took the participant to walk three metres (Phillips et al., 2013). This suggests that although this hormone ratio was not the link between depression and the immune changes we observed in participants with hip fracture, it may have a role to play in the markers of frailty we measured, at least in walking speed.

3.8 Circulating cytokine levels in hip fracture patients with depressive symptoms

Next, on measuring serum cytokine (immune messenger) levels in participants, we observed that hip fracture patients with depressive symptoms also had higher levels of pro-inflammatory cytokines, which are associated with higher levels of inflammation in the body, including IL6 and TNFα. We also observed higher levels of anti-inflammatory cytokines, such as IL10, in the participants with depression compared with non-depressed participants and controls, but overall depression scores did not positively relate to these levels (Duggal et al., 2013a). It is therefore unlikely that these immune messengers were the underlying link between depressive symptoms and immune function changes in our subjects.

3.9 Reduced T cell numbers in hip fracture patients with depressive symptoms

Finally, in an attempt to examine the effect of physical and psychological distress on adaptive immune cells (immune memory), we found several differences in cell numbers. T-cell (so called as they mature in the thymus gland) numbers were significantly lower in our group of hip fracture patients with depressive symptoms compared with those without depression or healthy controls (Duggal et al., 2014b). T cells are composed of two main subsets, CD4 T cells (helper T cells that help other cells recognise pathogens) and CD8 T cells (cytotoxic T cells that kill infected cells

directly). However, on examining the percentage of $CD4^{+ve}$ T cells and $CD8^{+ve}$ T cells no significant differences were observed between our three groups, or the ratio of these types of cells, which is sometimes higher in older or particularly stressed groups.

3.10 Numerical and functional deficit in regulatory B cells in hip fracture patients with depressive symptoms

B cells, are a type of cell involved in recognising pathogens in the body and producing antibodies against them. There are also specific types that help to regulate the function of the immune system to prevent over activity which might result in autoimmune disease. In this study, there were no differences in the total numbers of B cells between the three groups. However, a significant decline was seen in percentage and absolute numbers of regulatory B cells in hip fracture patients with depressive symptoms compared with healthy controls and hip fracture patients without depressive symptoms (Duggal et al., 2015b). Further, participants with higher depressive symptoms (GDS score) had lower frequency of regulatory B cells (Duggal et al., 2015b). All of these immune, hormonal and physical function differences between the depressed and non-depressed participants at week 6 are summarised in Table 2.

Table 2: Main group differences

	Direction of Group Difference		
Geriatric Depression Scale Score*	Depressed	>	Not Depressed
Neutrophil phagocytosis	Depressed	=	Not Depressed
Neutrophil superoxide generation*	Depressed	<	Not Depressed
Monocyte phagocytosis	Depressed	=	Not Depressed
Monocyte superoxide generation*	Depressed	<	Not Depressed
NK cell %/numbers	Depressed	=	Not Depressed
NK cell killing*	Depressed	<	Not Depressed
Cortisol*	Depressed	>	Not Depressed
DHEA*	Depressed	<	Not Depressed
Cortisol:DHEAS ratio*	Depressed	>	Not Depressed
Pro-inflammatory cytokines*	Depressed	>	Not Depressed
T-cell %/numbers*	Depressed	<	Not Depressed
CD4/CD8 T cell numbers/ratio	Depressed	=	Not Depressed
Regulatory B cell %*	Depressed	<	Not Depressed
Regulatory B cell cytokine production*	Depressed	<	Not Depressed
Oxford Hip Score*	Depressed	<	Not Depressed
Hand Grip Mean (kg)	Depressed	=	Not Depressed
Timed-Up-and-Go (seconds)*	Depressed	>	Not Depressed
Berg Balance Scale Score*	Depressed	<	Not Depressed
Length of hospital/rehab centre stay*	Depressed	>	Not Depressed

*Denotes significant differences between the groups at week 6

3.11 Long-term effect of depressive symptoms on hip fracture patients.

Finally, the long term effect of depressive symptoms on hip fracture patients was evaluated six months post-surgery. We observed that during the six month follow-up, neutrophil superoxide production had improved in both hip fracture groups, but monocyte and NK cell functioning in hip fracture patients with depressive symptoms still remained suppressed six months post-surgery. Further, the serum cortisol:DHEAS ratio remained high in the depressed group of hip fracture patients six months post hip surgery. This was not influenced by any change in reported depressive symptom scores at six weeks and six months, in fact there was very little change over time between the groups; six individuals who were not originally classified as depressed, were depressed at month six, and eleven who were initially depressed had recovered by month six. However, the biggest groups by far were those who had been depressed and remained so, and those who were not depressed and remained so.

3.12 Pilot study results

Next, our Punjabi sample was compared to our UK-based group of hip fracture patients on basic characteristics. The Punjabi sample was somewhat younger than the Caucasian sample, and there were more males. However, levels of anxiety and depression and activities of daily living scores were comparable across the samples. From the BIPQ, the Indian Punjabi group reported that they were significantly more affected by their hip fracture, perceived that the treatment had helped their hip fracture far less, and reported significantly more emotional distress resulting from their hip fracture compared to the UK sample. These differences are shown in Table 3 and were not driven by age and sex differences between the samples. Further, the Indian Punjabi group also reported higher levels of understanding about their hip fracture, but this became non-significant when taking age and sex differences into account in statistical analyses.

Table 3: Demographic and questionnaire pilot data in both samples

	Mean (Standard Deviation) or Number (%)	
	UK	India
Age	84 (7.96)	71 (9.76)
Sex (Male)	20 (20%)	11 (50%)
Activities of Daily Living	27.5 (9.24)	22.5 (9.57)
HADS Depression Score ≥ 8	26 (27%)	8 (36%)
HADS Anxiety Score ≥ 8	29 (30%)	5 (23%)
How much does your hip fracture affect your life?	6.0 (2.63)	7.45 (3.16)
How long do you think your hip fracture will continue?	4.9 (2.63)	4.1 (3.29)
How much control do you feel you have over the recovery from your hip fracture?	5.5 (2.9)	6.4 (3.4)
How much do you think your treatment helped your hip fracture?	7.4 (2.7)	1.6 (2.11)
How much do you experience symptoms from your hip fracture?	4.3 (2.65)	3.7 (3.62)
How concerned are you about your hip fracture?	4.6 (3.07)	5.8 (4.24)
How well do you feel you understand your hip fracture?	5.4 (3.5)	7.4 (3.5)
How much does your hip fracture affect you emotionally?	3.9 (3.16)	6.8 (4.08)

Scores ranges from 10 = high or best to 0 = low or worst.

All of the participants in both groups had fallen, but the reasons for the fall were perceived differently between the ethnic groups. The main cause provided by the UK Caucasian participants was that the hip fracture was due to the way that they fell (22%). This cause was not perceived by any of the Indian Punjabi participants, who attributed the main cause to be ‘fate’ (41%). However, difficulties in answering this open-ended question about the reason for the hip fracture in the Punjabi sample meant that this question was followed up with an additional probe: “Some people think that their fracture was due to fate or bad diet, do you think any of these factors caused your illness?” This is likely to have introduced some bias into the distribution of answers across the samples. Other perceived causes of hip fracture are shown in Table 4.

Table 4: Participants' perceptions of causes of hip fracture

Perceived cause of hip fracture	Number (%)	
	UK	India
The way they fell and landed	22 (22)	0 (0)
Bone related cause (osteoarthritis or low calcium levels)	9 (9)	1 (4.5)
Blamed themselves (e.g. for being careless)	7 (7)	0 (0)
Dizziness	4 (4)	1 (4.5)
Age	4 (4)	2 (9)
Fate	0 (0)	9 (41)
No cause known/given	55 (55)	9 (41)

4.0 Implications in terms of policy and practice

The clear implications arising from this research is that depression in older adults post-hip fracture is not only common, but can have a range of catastrophic consequences including poorer immunity across a whole range of immune measures, worse physical frailty and recovery, and longer stays in NHS facilities. Our findings support the need for preventing and treating depression and depressive symptoms to improve outcomes in older people with hip fracture, and that this should become common practice. One particular difficulty that this immediately raises is that patients with hip fracture are not routinely screened for depression symptoms, thus we would recommend this should be integrated into normal clinical practice. The use of a short scale such as the Geriatric Depression Scale would not add significant workload but would identify those patients at risk of poorer outcomes. Interestingly, as well as group differences, our findings also showed that all immune outcomes were worse among those with the highest depression scores, meaning that for some individuals, the effects of untreated depressive symptoms post-fracture are very severe. Poorer immune outcomes means that these patients are more at risk of infection (Butcher et al., 2005), which often results in mortality or at least return to hospital. Poor physical function outcomes means that these patients lose independence and are more likely to need to be admitted to rehabilitation facilities, or may not ever be fit enough to return home. Consequently, it is of great importance to identify such patients in terms or improving their health and wellbeing as well as reducing NHS costs in the longer term. Use of such a measurement would also indicate those patients struggling most emotionally after the physical trauma of their fracture, and thus identify those most in need of supportive care for up to six months post-fracture.

There are also a number of obvious routes for intervention in these patients. Although participants' GPs were informed of their involvement in the study and high depressive symptoms scores, this resulted in only two participants being prescribed anti-depressive medication. This may reflect that patients' hip fracture is the main cause for concern and focus of treatment at this time, or also that common anti-depressant medications are contra-indicated in frail older adults due to interactions with osteoporosis and the risk of further serious injury. However, the difficulties inherent in not assessing and treating such symptoms are outlined above, thus it is likely that some type of psychological or pharmaceutical depression treatment would be helpful for these patients not only in terms of psychological wellbeing but also in terms of physical health and day to day functioning.

The link with the cortisol:DHEAS ratio in the present study also suggests another route for intervention. As a higher ratio was related to poor physical frailty and slower walking speed recovery, it is possible that an intervention to adjust this ratio would have beneficial effects on recovery. DHEA tablets given as a nutritional supplement have been shown previously to be effective at increasing wellbeing and mood in older adults (Arlt et al., 2000, Arlt et al., 1999), improving some measures of physical function (Buvat, 2003), as well as being safe and well tolerated at doses of 50mg (Arlt et al., 1998). Further, DHEA supplementation is also known to modify immune function (Arlt and Hewison, 2004), for instance an immune enhancing effect of DHEAS has been reported on neutrophil superoxide generation (Radford et al., 2010) and NK cell cytotoxicity (Solerte et al., 1999, Casson et al., 1993). Although some studies have shown limited effectiveness in healthy populations, we believe the real effects of DHEA supplementation are likely to be seen among those with adrenal insufficiency or at acute risk of a high cortisol:DHEAS ratio, such as in our elderly depressed hip fracture patients. DHEA is also a cheap nutritional supplement, thus would not cost as much as testing as introducing a new anti-depressant that is safe among older adults, or time-intensive psychological therapies. It is also possible that such treatment could be combined with some type of psychological support such as social support or talking therapy for older adults with depressive symptoms, which might result in even more positive health and wellbeing outcomes. Further research on the likely effectiveness as well as cost-effectiveness of such interventions is our next plan.

The implications of our pilot research are limited by the inability to identify and recruit sufficient UK-based Punjabi patients. This reflects both low numbers of this group having hip fracture as well as difficulty with recruitment, even with a Punjabi speaking research assistant. Given this, our data are based on Punjabi patients in India where time since hip fracture and age of patients differed to our UK sample, making it difficult to generalise this group to a Punjabi patient group in the UK. However, it was interesting to note that Punjabi patients felt more affected and less effectively treated than UK Caucasian patients, and it would be interesting to conduct further research to see whether this might be the case also within the UK National Health Service or is specific to India. Further, Punjabi patients were more likely to attribute their hip fracture to fate, whereas UK Caucasian patients attributed it to the way they fell. However, caution should be

taken here, as the questionnaires were conducted as interviews in the Punjab, and when answers were not forthcoming, the research assistants gave prompts based on previous patients' answers, which are likely to have biased self-reports. However, if further research were to replicate this finding without prompting from the researchers, it would raise interesting questions regarding patients' understanding of illness and reasons for illness within the health care system in India, suggesting that more communication and education about the source of illness and reasons for treatment might be necessary. This would be important as illness perceptions can affect patient engagement with the healthcare system and rehabilitation, with consequent effects on patient health and wellbeing outcomes.

5.0 Conclusion summarising the key points, highlighting significance for ageing research, policy and practice, and indicating the next steps for research.

In conclusion, this NDA project reported for the first time that development of depressive symptoms in older people who have suffered hip fracture can result in suppressive effect of psychological distress of depressive symptoms on neutrophil, monocytes and NK cell functioning in older hip fracture patients. Additionally, certain aspects of immunity in the participants with hip fracture and new onset depression remained suppressed even six months post-surgery. Further, the development of depressive symptoms post hip fracture induces HPA axis activation, resulting in elevated cortisol levels in people with hip fracture patients and depressive symptoms. Additionally, a reduction in serum DHEAS levels were also observed in these participants, resulting in an overall elevated serum cortisol: DHEAS ratio in hip fracture patients with new onset depression that remained elevated even six months post-surgery. We have also reported a reduced frequency of circulating B regulatory cells in hip fracture patients with depressive symptoms. These findings suggest that development of depressive symptoms after a hip fracture in older adults is the main driver of immune suppression, as we failed to find an immune decline as a result of hip fracture alone. Further, depression emerging post-hip fracture in older adults impaired physical function, including walking speed, balance, and activities of daily living. This showed for the first time that depressive symptoms in the absence of longer term pre-fracture depression diagnosis relate to recovery and physical frailty. Effects on walking speed were mediated by alterations in the cortisol:DHEAS ratio which was heightened among the depressed group. Finally, the adults with hip fracture who developed depression were also more likely to spend longer in NHS facilities overall, incurring significant cost to the health service as well as inconvenience to patients not well enough to return home.

This novel finding implies that in order to speed recovery of physical function, immunity and infection protection, and independence following hip fracture, patients should be assessed and treated for depressive symptoms. This is of relevance to surgeons and health professionals alike involved in rehabilitation post-fracture surgery who currently do not screen this patient group for depressive symptoms. Identification and treatment of depression in these patients would improve patient outcomes and quality of life as well as impacting upon health service costs incurred

through treatment of those with slower recovery and decreased independence post-fracture. We propose that correcting the cortisol:DHEAS imbalance by oral supplementation with DHEA may be one means of improving depressed mood and contributing to better physical function after hip fracture. However, such an intervention would need to be cautiously informed by the intervention literature in order to determine an effect dosage and regime for an effect in these patients. It is possible that some form of psychological support on top of DHEA treatment might result in the best outcomes for patients who have developed depressive symptoms. Consequently, the next stage in our research will be to pilot a randomized controlled trial of DHEA supplementation with and without psychological support in older adults with hip fracture and depression and ascertain effects on wellbeing, physical frailty, and immunity.

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